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Anti-Brownian Traps

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Synonyms

ABEL trap; Single-molecule methods

Introduction

Brownian motion is typically considered an inescapable attribute of small particles in free solution. This random jiggling often impedes or prohibits optical studies of the behavior of nanometer-sized objects, such as single biomolecules, because such objects quickly diffuse away from the observation region. The rate of diffusion increases with decreasing particle size, so the window of opportunity for measuring small particles is very short. Anti-Brownian traps address this challenge by partially suppressing Brownian motion: the position of a single particle is monitored, and active feedback is used to apply forces that directly counteract the observed displacements. This process confines the particle to a small region of interest (ROI), enabling extended study without surface attachment or encapsulation, both of which could perturb the particle's behavior. A powerful single-molecule method, anti-Brownian traps have been used to hold particles ranging in size from single small organic fluorophores and ~10 nm biomolecules (Fields and Cohen 2011) to human cells (Armani et al. 2006). Several different methods of tracking and sources of feedback have been successfully employed to trap particles in various geometries. While trapped, a wide range of imaging and spectroscopic probes may be employed to extract multiparametric information from the trapped object as in single-molecule spectroscopy (Fig. 1).

Tracking Particle Position

Diffusion of Particles

Thermally agitated solvent molecules undergo random collisions with any particle (molecule or colloid) immersed in the solution. In the absence of other forces, these collisions cause the particle to undergo a random walk with a mean square displacement along each axis that increases linearly with time

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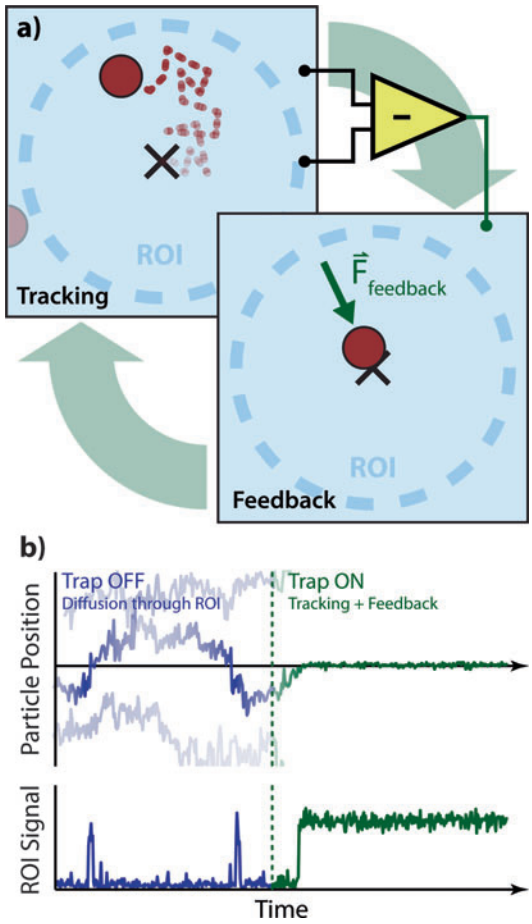


Anti-Brownian Traps, Fig. 1 Schematic operation of an anti-Brownian trap. (**a**) Feedback principle underlying anti-Brownian traps. The position of a single particle diffusing within an observation region of interest (ROI) is monitored (*left*). Feedback is applied in an active control loop to correct the position of the particle relative to the target (*right*). This cycle is repeated as fast as possible to maintain the particle within the ROI. (**b**) Expected patterns of particle displacement (top) and optical signal (usually fluorescence) from the ROI (bottom). Without trapping (dark blue), particles diffuse randomly through the ROI, with very short residence times and brief bursts of signal. Once the trap is turned on (green), a single particle remains trapped in the ROI, producing a high signal for a long time

$$\langle D^2 \rangle = 2Dt. \tag{1}$$

The diffusion coefficient, D, is related to the properties of the particle and the solution by the Stokes-Einstein formula:

$$D = \frac{k_B T}{6\pi\eta a}, \tag{2}$$

where k_B is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the solution, and a is the hydrodynamic radius of the particle (equal to the physical radius for a spherical particle). A nanometer-scale particle will diffuse out of a micron-scale region in just a few milliseconds, which is an insufficient observation window to capture many biological processes or to provide precise measurements. Expanding the region of interest might prolong the observation window but only at the cost of a decreased signal-to-background ratio.

Implementation of Particle Tracking

A variety of tracking systems have been employed in anti-Brownian traps. The conceptually simplest systems use a video camera and particle-tracking software, paired with either fluorescence or bright-field imaging, to track the position of a particle in 2-D (Armani et al. 2006; Cohen and Moerner 2005, 2008). Camera-based systems are flexible and relatively simple to set up, but the feedback bandwidth is often limited by the electronics in either the camera or computer.

More recent trap designs have employed a laser scan pattern in 2- or 3-D paired with time-resolved detection at a single point sensor, much like in confocal microscopy, to estimate the position of the particle (Fields and Cohen 2011; Cohen and Moerner 2008; Berglund and Mabuchi 2004; Berglund et al. 2007; Goldsmith and Moerner 2010; Wang and Moerner 2010). In this detection scheme, photons may originate from either fluorescence or scattering of the trapped object. Alternatively, multiple point detectors in the image plane may be used to monitor a small number of locations in and around the target ROI, providing fast position estimation in 2- or 3-D without the added complexity of coordinating a laser scan with photon arrival times (Lessard et al. 2007; Cang et al. 2007). Most Anti-Brownian traps use one-photon fluorescence both for the tracking and for simultaneous spectroscopic measurements. However, tracking systems based on two-photon

fluorescence (Levi et al. 2005) and scattering (Cang et al. 2006) have also been demonstrated. Due to the statistical independence of the Brownian motion of distinct objects, anti-Brownian trapping of two or more objects requires independent force feedback systems for each object and cannot readily bring two trapped objects together.

Optimization of Particle Tracking

The optimal bandwidth of a particle tracking system is set by a balance between diffusion and shot noise-limited localization precision. Tracking errors due to diffusion grow as the rms displacement $\sqrt{2D\tau}$, where τ is the measurement integration time. Tracking errors due to shot noise shrink as $\sigma/\sqrt{\Gamma\tau}$, where σ is the localization error for a single photon and Γ is the photon detection rate. Tracking errors are minimized when these two errors are approximately equal, corresponding to an integration time of $\sigma/\sqrt{2D\Gamma}$ (Fields and Cohen 2012).

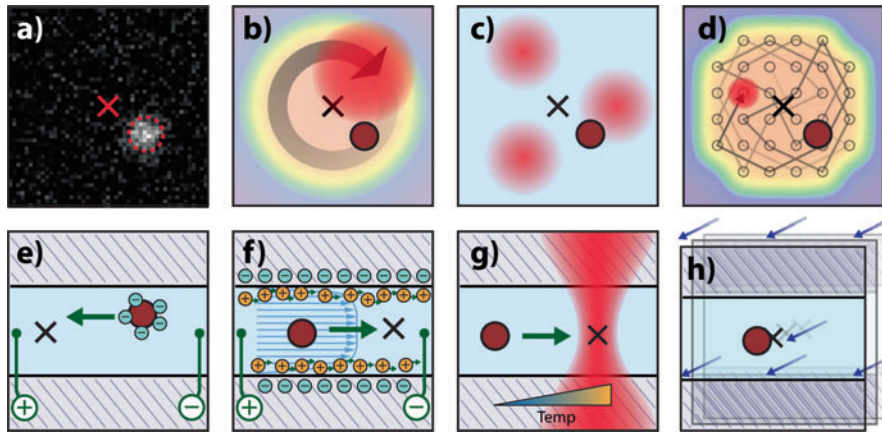
Since smaller objects have larger diffusion coefficients, the tracking system of an anti-Brownian trap must locate a particle quickly and accurately enough to enable stable closed-loop control over the mean particle position. The algorithm used to track the particle depends upon the detection scheme.

For video tracking, the particle position estimate is updated once per frame and may be as simple as locating the peak or centroid of the particle's image spot (point spread function, PSF). However, the minimum frame time of many camera-based systems exceeds the optimal integration time for tracking small particles. Extension of image-based trapping to 3-D traps requires differentiation of positions above and below the image focus, for example, by use of an astigmatic PSF (King et al. 2013).

Detection schemes that rely on a laser scan pattern and a single point detector encode the particle position using the deterministic behavior of the scan pattern. Because the position of the excitation spot is known as a function of time, the pattern of photon arrival times directly reveals the location of the particle. In this case, the position estimate for the particle may be updated as often as single photons are detected, though due to the finite accuracy of each photon localization, there remains merit in combining information from multiple photons. For certain scan patterns, such as an annulus or spatially separated excitation spots, the time-averaged excitation intensity is not spatially homogeneous in and around the trapping region, and therefore the time-dependent fluorescence intensity may provide additional information about the particle position relative to the scan pattern (Levi et al. 2005; Enderlein 2000; Berglund and Mabuchi 2005). More optimal scan patterns are grid-based, maximizing the position information encoded in each photon while minimizing the expected interval between successive photon detection events and providing nearly flat time-averaged illumination over the entire trapping region. Combining these scan patterns with a more sophisticated algorithm, such as a Kalman filter, to recursively update the position estimate (using reasonable assumptions about the particle's dynamics) can reduce the influence of background photons that do not originate from the trapped particle and can improve feedback decisions (Wang and Moerner 2010; Fields and Cohen 2012). Multiple point detectors can also be used to infer the position of the particle when combined with several fixed excitation spots (Cang et al. 2007). This strategy also works in 3-D with four spots placed at the corners of a tetrahedron (Lessard et al. 2007) (Fig. 2).

Trapping Via Active Feedback

One must exert forces on a molecule to counter its Brownian motion. Anti-Brownian traps most commonly employ electrokinetic feedback to manipulate the position of a particle, as in the Anti-Brownian ELectrokinetic trap (ABEL) trap (Cohen and Moerner 2005; Wang et al. 2012). Force is generated through electric fields produced by applying voltages to a sample contained within a micro- or nano-fabricated sample cell. These fields generate electrophoretic and electroosmotic forces that together move the particle. The relative contributions of electrophoresis (where the field produces force through the



Anti-Brownian Traps, Fig. 2 Implementation strategies for position sensing and feedback in an anti-Brownian trap. As described in the text, tracking strategies include (**a**) video tracking with a camera, (**b**) rotating beam, (**c**) multiple point detectors, and (**d**) timed scan pattern with a single point detector. Feedback strategies (*bottom row*) include (**e**) electrophoresis, (**f**) electroosmosis, (**g**) thermophoresis, and (**h**) stage motion feedback

particle's charge) and electroosmosis (where the field moves the entire liquid via motion of surface counterions) depend on the charge of the particle, the mobile surface charge within the trap, and the geometry of the trap. Electrokinetic feedback is sufficiently fast to trap individual fluorophores in water (Fields and Cohen 2011; Wang and Moerner 2012) but is limited to homogeneous solutions of low to moderate ionic strength (< ~100 mM). Furthermore, fabrication of microfluidic sample cells can be easy if PDMS is used or challenging if quartz is required for purposes of low fluorescence background.

Flow-based active feedback to reposition a trapped particle need not be induced only by electroosmosis. Simple hydrostatic pressure actuators can regulate flow through a microfluidic device, moving the location of the stagnation point to trap small particles using active control (Tanyeri et al. 2011; Ropp et al. 2010). While these devices are highly sensitive to the precise geometry of the microfluidic cell and valve actuators, one additional advantage is the ability to impart shear force on a trapped object.

Recently, it was shown that thermophoretic forces can also be used to actively control the position of a particle (Braun and Cichos 2013). In this approach, a laser is used to selectively heat a spatial pattern in or near the trap, setting up a temperature gradient that can generate a net drift of a particle either toward or away from a heat source due to the thermodynamic effects on that particle's entropy.

Rather than applying force to move the particle, it is also possible to simply move the ROI to follow the particle's position (Berglund and Mabuchi 2004; Lessard et al. 2007; Levi et al. 2005; Cang et al. 2006; Lu et al. 2007). This goal may be achieved in two different ways: translating the entire sample to move the particle back to the ROI or moving the ROI within the sample. In translation-based feedback, the entire sample is translated to keep the trapped particle approximately stationary in the laboratory frame of reference and centered in the optical collection path. This approach is typically implemented with a piezoelectric translation stage in two or three dimensions. Stage motion allows long-time observation in complex environments such as cells but is typically too slow to trap very small objects in aqueous buffer. Alternatively, the ROI can be optically translated to follow the diffusing particle. Neither of these approaches repositions the particle relative to objects in its immediate environment nor can they provide information on the charge state of the particle, and the probability of encountering dirt particles or conditions that

interfere with trapping rises with increased range of exploration.

Recent reviews (Fields and Cohen 2010; Banterle and Lemke 2016) provide an excellent overview of the technical capabilities and limitations of different approaches to implementing feedback in an anti-Brownian trap. The original anti-Brownian traps held single particles within a two-dimensional region, but later designs extended the concept to multiple particles (Armani et al. 2006) and to three dimensions (Berglund et al. 2007; Cang et al. 2006; King et al. 2013; Wells et al. 2010).

Multiparametric Monitoring of Trapped Particles

The selection of a tracking method is directly influenced by the type of data that is to be acquired. A maximally efficient anti-Brownian trap will use the same photons for feedback and to learn about the trapped particle. For example, with fluorescently labeled samples, photons arriving at the detector may be used to determine the particle's position, and changes in brightness, fluorescence lifetime, spectrum, or polarization anisotropy may report on the state of the molecule at any given moment in time (Wang and Moerner 2012). As implementations of anti-Brownian trapping have evolved, it has become possible to track many spectroscopic properties simultaneously, giving unique long-timescale insight into changes in the photophysical state of the trapped particle (Squires and Moerner 2017).

The capability to monitor multiple spectroscopic variables has also enabled more complicated analyses to be performed on the fly, such as real-time estimation of a trapped particle's electrokinetic mobility and diffusivity which can report on charge and size (Wang and Moerner 2015). These physical variables can be used to quickly classify the physical state of the trapped object, such as aggregation or hybridization, even when these states might not be obvious from optical parameters (Fig. 3).

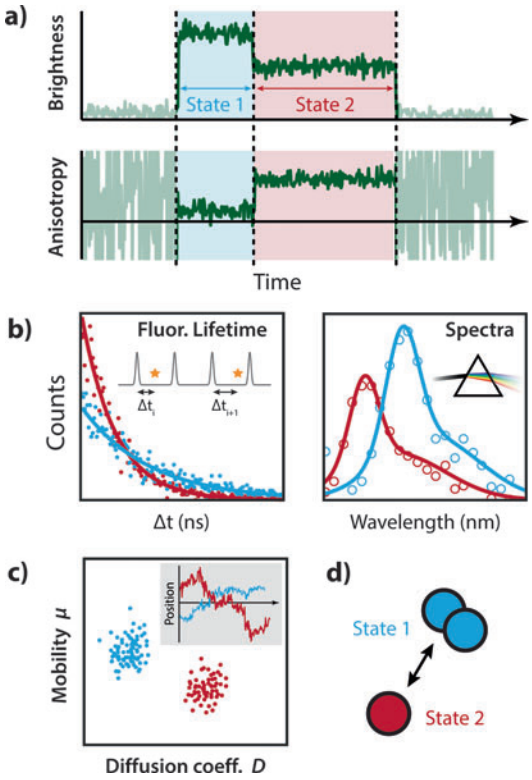
Comparison to Passive Trapping and Confinement

Single particles can be spatially confined without the use of active feedback, by encapsulation in a nanochannel, nanocavity, gel, or vesicle. As with tethering a particle to a surface or bead, the primary concern is that the function or aspect of interest will be altered by these physical constraints that alter the local environment.

Popular passive trapping schemes, including optical tweezers, magnetic tweezers, and dielectrophoresis, require a local minimum in the potential. This minimum is difficult to generate for nanoscale particles because the trapping strength of these techniques relies on the polarizability of the trapped object, which scales roughly with volume. So, particles smaller than 100 nm in diameter cannot be trapped with biologically safe laser powers or voltages. Anti-Brownian traps typically outperform these passive trapping approaches for trapping particles smaller than 100 nm all the way down to 1 nm. Passive hydrodynamic and thermophoretic traps can also trap very small particles but cannot prevent the accumulation of multiple particles at the trap minimum.

Applications

Applications of anti-Brownian traps range from basic physics through chemistry, biochemistry, and biology, from the nanoscale up to the microscopic behavior of individual cells. A wide range of natively fluorescent and fluorescently labeled biomolecules have been studied, including the polymer dynamics (Cohen and Moerner 2007) and hybridization (Wang and Moerner 2014) of DNA, the photophysics and aggregation of phycobiliproteins such as allophycocyanin (Goldsmith and Moerner 2010; Wang and Moerner 2015; Squires and Moerner 2017), photodynamics and quenching in antenna complexes (Bockenhauer and Moerner 2013; Schlau-Cohen et al. 2013, 2015), and conformational shifts in, e.g., G protein-coupled receptors (Bockenhauer et al. 2011). These traps have also been used to



Anti-Brownian Traps, Fig. 3 Multiparametric detection of a trapped particle. Illustration of a case revealing two distinct photophysical states (1, blue, 2, red) of a hypothetical fluorescent or fluorescently labeled sample: (**a**) brightness and anisotropy, (**b**) fluorescence lifetime and emission spectra, (**c**) mobility parameters μ and D. Monitoring all parameters simultaneously provides rich spectroscopic data, which can suggest a hypothetical model (**d**) for the system

observe the dynamics of the TRiC/CCT multisubunit enzyme involved in protein folding by counting the number of ATP molecules on each enzyme (Jiang et al. 2011) and single electron transfer events in nitrite reductase (Goldsmith et al. 2011). Nearing the macroscopic scale, anti-Brownian traps have even been used to monitor internalization of particles by cells (Wells et al. 2010; Welsher and Yang 2014).

Anti-Brownian traps have also been employed to observe more basic physical phenomena, including orientation-dependent scattering in metallic nanostructures (Cang et al. 2008), detection of the electron spin resonance of ~30 nm nitrogen vacancy centers (Kayci et al. 2014), determining the dynamical effects of virtual potentials (Jun and Bechhoefer 2012), and performing a high-precision test of Landauer's principle (Jun et al. 2014). More details on these and other applications can be found in recent reviews (Wang et al. 2012; Schlau-Cohen et al. 2014).

Summary

Anti-Brownian traps make it possible to keep a single particle within a very small region of interest in free solution for seconds or even minutes, without the need to physically confine or tether it. The key to canceling out the Brownian motion of a particle is to track its position quickly and accurately and to apply a compensating force in response to each detected position that will push the particle back toward the center of the trap. A variety of tracking and feedback approaches have been successfully implemented to trap and observe single particles while monitoring a growing list of physical and spectroscopic properties in real time, highlighting the versatility of this single-molecule confinement and measurement technique.

Cross-References

- Optical Tweezers
- Single Particle Tracking
- Single-Molecule Methods
- Single-Molecule Spectroscopy

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